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ORIGINAL RESEARCH

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The analysis of contrast-enhanced ultrasound features of the liver in male patients with liver cancer and unhealthy life habits under 50 years-of-age

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Abstract

To analyze the contrast-enhanced ultrasound (CEUS) features of livers in male patients under 50 years-of-age with liver cancer and unhealthy life habits. A total of 89 male patients with liver cancer and unhealthy life habits were included in the observation group (all of these patients were first diagnosed with liver cancer when they were <50 years-of-age) and 60 male patients of the same age with benign liver lesions admitted during the same period were included in the control group. All patients had undergone CEUS examination. We summarized and compared the features of liver CEUS from patients in the two groups and analyzed the relationship between liver CEUS results and pathological features. The time to enhancement, enhancement duration and the clearance time of cancer tissues were all significantly shorter in patients from the observation group than those in the control group ($p < 0.05$). Enhancement patterns of cancer tissues and benign lesion tissues in patients in the observation group were significantly higher than those in the control group ($p < 0.05$). Cancerous tissues had fast wash-in and fast wash-out features (93.2%), while benign lesions had slow wash-in and slow wash-out features (60.00%). The proportion of patients with microvascular invasion (MVI)-positive lesions showing more than a 15% increase in maximum diameter was significantly larger than those with MVI-negative lesions ($p < 0.05$). The differences in enhancement features between liver cancer lesions with various differentiation grades in the portal phase and the delayed phase were statistically significant ($p < 0.05$). Our findings show that CEUS has high clinical value for diagnosing liver cancer and can be used to judge the MVI status and differentiation grade of liver cancer lesions, thus providing a reference for diagnosing liver cancer and its severity.

Keywords

Male; Liver cancer; CEUS; Pathological feature

1. Introduction

Recent statistical evidence demonstrates that the majority of patients with liver cancer are men, and that the ratio of males to females who are diagnosed with liver cancer is 3.5 to 1. This finding is related to the fact that unhealthy life habits, such as smoking and drinking, are more common in males than in females [1, 2]. Liver cancer is a common malignant tumor of the digestive system that develops rapidly and has a high mortality rate in its later stages. The early diagnosis of liver cancer is important as this can permit timely treatment measures and improve the prognosis of patients [3]. Contrast-enhanced ultrasound (CEUS) can effectively enhance contrast signals from imaging and can clearly visualize changes in blood flow and perfusion status in a qualitative diagnostic manner; this is achieved by blasting and vibration of a contrast agent [4]. The liver has an abundant blood supply. However, patients with liver cancer have an abnormal blood supply; consequently, the blood supply features of a liver affected by cancer are obviously different from those of normal liver tissue [5, 6]. Evidence has shown that CEUS can clearly visualize the vascular anatomy of liver tumors and evaluate the physiological function of tumors [7]. In this study, we summarize the CEUS features of males with liver cancer who adopt unhealthy life habits. Our aim is to provide reference guidelines for the diagnosis of liver cancer by CEUS.

2. Objects and methods

2.1 Objects

A total of 89 male patients with liver cancer and a history of unhealthy life habits were included in the observation group (all of these patients were first diagnosed with liver cancer under 50 years-of-age) and 60 male patients of the same age with benign liver lesions who were admitted during the same

period were included in the control group.

The inclusion criteria were as follows: (1) patients who drank or smoked for a very long time (excessive drinking refers to drinking more than 60 g of spirits every day for more than 1 year; a long history of smoking refers to smoking more than 10 cigarettes every day for more than 1 year); (2) patients who were younger than 50 years-of-age; (3) patients who had been diagnosed with hepatitis B for longer than 5 years or were carriers of the hepatitis virus for more than 5 years; or patients who had a history of chronic hepatitis, or a family history of liver cancer, cirrhosis or long-term exposure to substances that can cause liver damage, such as a severe fatty liver or abnormal alpha-fetoprotein (AFP) levels; (4) patients who had a CEUS examination less than one month before surgery and had a complete set of imaging data; (5) patients who did not receive radiotherapy or chemotherapy prior to surgery; (6) patients who had only one lesion; (7) patients whose pathological results were clearly confirmed by biopsy under ultrasonic guidance at the time of surgery.

Patients were excluded if they had an incomplete set of clinical data or results showing poor image quality. Female patients were excluded along with patients diagnosed with other malignant tumors. We also excluded patients who were allergic to contrast agent.

2.2 CEUS examination

(1) Instruments and reagents: An Acuson Sequoia 512 system (Acuson Sequoia 512, Siemens, Germany), equipped with a 4C-1 probe was used to generate abdominal CEUS sequences *via* CEUS contrast pulse sequencing technology. A PhilipsiU22 ultrasonic apparatus (PhilipsiU22, Amsterdam, Philips, Netherlands) with a probe frequency of 1~5 MHz was used and a low mechanical index, anti-pulse, reverse harmonic imaging technique was adopted. SonoVue (BR-1, Bracco, Milan, Italy) was used as the contrast agent.

(2) Methods: The patient was asked to lie on his/her back or side while exposing the body region to be examined. Then, the physician adjusted the probe and instrument parameters to clearly visualize the required details and surrounding liver tissues. Conventional ultrasound was used in a restricted manner to scan the entire liver; this allowed us to record the position, size, echo, boundary of the lesion, and hemodynamic changes of the inner portion and peripheral tissues of the lesion. The probe was fixed on the rear. Then, the physician selected contrast mode and adjusted the corresponding parameters to provide the ultrasound instrument with a low mechanical index. Finally, only the harmonic signal of the contrast agent was displayed on the ultrasound instrument. Next, 5 mL of normal saline was injected into lyophilized SonoVue powder, a second-generation acoustic contrast agent. Once the powder had been dissolved, 2.4 mL of microbubble suspension was extracted and quickly injected manually through the peripheral veins. This was followed by an injection containing 5 mL of normal saline. The contrast agent was injected *via* a peripheral vein and a timer was initiated. The physician observed the injection process of contrast agent into the lesion and surrounding liver tissues in real time for at least 6 minutes; the entire process was recorded on a hard disk.

(3) Image analysis: Two sonographers with >5 years of clinical experience were then asked to analyze the images. The sonographers observed enhancement of the lesion and surrounding tissues in different phases and recorded the corresponding data. Analysis involved one gray scale image and one image showing the lesion and its anatomical position. The entire enhancement procedure of CEUS can be divided into an arterial phase, portal phase and a delayed phase. We analyzed several observation indices, including time to enhancement (the time at which contrast agent began to emerge after injection), the time to peak enhancement (the time at which the intensity of contrast agent reached peak levels in the lesion), clearance time (the time at which the intensity of contrast agent in the lesion started to fall lower than that in the surrounding liver tissues after injection), enhancement status (fast wash-in and fast wash-out, fast wash-in and slow wash-out, fast wash-in and simultaneous wash-out, fast wash-in and no wash-out, slow wash-in and fast wash-out, slow wash-in and no wash-out) and enhancement patterns (global enhancement and local enhancement, homogeneous enhancement and inhomogeneous enhancement).

2.3 Pathological examination

Following surgery, tissue samples were stained with hematoxylin and eosin. Hepatocellular carcinoma (HCC) cases were diagnosed according to the international consensus on hepatoma. Microvascular invasion (MVI) was defined as the presence of tumor cells within a vascular lumen lined by an endothelium that is visible only by microscopy. According to the World Health Organization (WHO), tumor differentiation was divided into low grade, intermediate grade and high grade [8].

2.4 Statistics

SPSS version 19.0 (Statistical Package for Social Sciences 19.0, IBM, Armonk, NY, USA) was used for data processing. Measurement data was expressed as mean ± standard error and the means between the two groups were compared by the *t* test. Numerical (count) data were expressed by cases and the χ^2 test was used for comparisons between the two groups. $p < 0.05$ was statistically significant.

3. Results

3.1 Enhancement time

The time to enhancement, enhancement duration and clearance time of cancer tissues from patients in the observation group were all significantly shorter than those in the control group ($p < 0.05$; Table 1).

3.2 Comparison of enhancement methods

There were significant differences between cancer lesions and benign lesions in terms of enhancement methods ($p < 0.05$). Cancer lesions showed as fast wash-in and fast wash-out (93.26%) while benign lesions showed as slow wash-in and slow wash-out (60.00%). Further details are given in Table 2.

TABLE 1. Comparison of enhancement time (mean ± SD).

Group	n	Time to enhancement	Enhancement duration	Clearance time
Observation group	89	20.15 ± 3.25	45.55 ± 10.36	66.58 ± 7.85
Control group	60	35.57 ± 4.49	177.58 ± 16.69	240.15 ± 13.66
t		24.314	59.570	98.280
p		<0.001	<0.001	<0.001

TABLE 2. Comparison of enhancement methods (n (%)).

Group	n	Fast wash-in and fast wash-out	Fast wash-in and slow wash-out	Fast wash-in and simultaneous wash-out	fast wash-in and no wash-out	slow wash-in and fast wash-out	slow wash-in and slow wash-out
Observation group	89	83 (93.26)	3 (3.37)	1 (1.12)	0 (0.00)	1 (1.12)	1 (1.12)
Control group	60	2 (3.33)	15 (25.00)	5 (8.33)	2 (3.33)	0 (0.00)	36 (60.00)
χ^2				122.977			
p				<0.001			

3.3 The sensitivity, specificity and accuracy of CEUS for the diagnosis of liver cancer

The sensitivity, specificity and accuracy of CEUS for the identification of liver cancer were 91.67%, 95.51% and 93.96%, respectively (Table 3).

3.4 The relationship between CEUS intensity, conventional ultrasound and MVI

In CEUS, the maximum diameter of a liver cancer lesion was defined as the maximum diameter of a lesion at its arterial peak; we compared this parameter with that obtained by conventional ultrasound. Patients for which the maximum diameter of a lesion in CEUS examination increased by >15% were included one group, while those for which the maximum diameter increased no more than 15% were included in another group.

We found that the proportion of patients with MVI-positive lesions with a maximum diameter increasing by >15% was significantly larger than those with MVI-negative lesions ($p < 0.05$). Further details are given in Table 4.

3.5 The correlation between CEUS intensity and tumor differentiation

There were 69 liver cancer patients with a low/intermediate grade of differentiation and 20 patients with a high grade of differentiation. The differences in enhancement features between liver cancer lesions with various differentiation grades in the portal phase and delayed phase were statistically significant ($p < 0.05$; Table 5).

4. Discussion

Hepatitis B virus (HBV) infection, aflatoxin-contaminated food and genetic factors are all known risks for liver cancer. However, the adoption of unhealthy life habits, such as drinking and smoking, are also known to increase the risk of liver cancer [9–11]. In the clinic, many patients with liver cancer have a history of smoking or drinking. Conventional ultrasound has always been the first choice for the clinical screening of liver cancer, and can effectively visualize the size, number, position, internal echo and other parameters, in liver cancer lesions. However, ultrasound can be affected by a range of factors, including acoustic window, gas, body position and angle; thus, conventional ultrasound is of limited value for the diagnosis of liver cancer [12, 13]. Research has shown that CEUS can comprehensively and objectively reflect blood perfusion in new vessels and micro-vessels within tumors, thus improving the clinical diagnosis of liver cancer [14–16].

The liver has an abundant blood supply; 70%–75% of hepatic blood comes from the portal veins, while 90% of the blood supply to liver cancer cells comes from the hepatic arteries; this indicates that the development of liver cancer involves a change in hepatic blood supply. With the exacerbation of nodules, the blood supply in the hepatic arteries gradually increases and the neoplastic vessels gradually form a micro-vascular network, thus providing a pathological basis for the screening of liver cancer [17–19]. In the present study, compared to benign nodules, we found that liver cancer lesions had a shorter time to enhancement, a shorter enhancement duration and a shorter time to clearance; most of the enhancement patterns were fast wash-in and fast wash-out (93.26%). One explanation for these findings is that most of the blood supply to liver cancer lesions originate from the hepatic arteries; however, the vascular structure in the lesions becomes disordered, irregular and distorted. Another potential reason is that these blood vessels may grow intensively with uneven sizes. Thus, after injection, contrast shows fast enhancement in tumors, and fast clearance in the advanced arterial phase [20–22]. These findings are related to the fact that the abundant blood supply of the hepatic artery and the formation of new blood vessels can accelerate micro-vascular perfusion and blood circulation, thus resulting in a shorter circulation time for contrast agents during the advanced arterial phase [23–25]. In this study, we also found that the sensitivity, specificity and accuracy of CEUS for the diagnosis of liver cancer were 91.67%, 95.51% and

TABLE 3. The sensitivity, specificity and accuracy CEUS for the diagnosis of liver cancer (n (%)).

CEUS examination	Pathological examination		Total	Sensitivity (%)	Specificity (%)	Accuracy (%)
	Benign	Malignant				
Benign	55 (93.22)	4 (6.78)	59 (39.60)	91.67	95.51	93.96
Malignant	5 (5.56)	85 (94.44)	90 (60.40)			
Total	60 (40.27)	89 (59.73)				

CEUS: contrast-enhanced ultrasound.

TABLE 4. Relationship between the intensity of CEUS, conventional ultrasound and MVI (n (%)).

MVI	Maximum diameter increasing by more than 15%	Maximum diameter increasing by no more than 15%	Total
Positive	22 (55.00)	18 (45.00)	40 (44.94)
Negative	10 (20.41)	39 (79.59)	49 (55.06)
χ^2	11.444		
p	<0.001		

MVI: microvascular invasion.

TABLE 5. The correlation between CEUS intensity and tumor differentiation (n (%)).

Differentiation grade	Portal phase		Delayed phase		Total
	Low enhancement	High/equal enhancement	Low enhancement	High/equal enhancement	
Low/intermediate grade of differentiation	49 (71.01)	20 (28.99)	60 (86.96)	9 (13.04)	69 (77.53)
High grade of differentiation	6 (30.00)	14 (70.00)	11 (55.00)	9 (45.00)	20 (22.47)
χ^2	11.049		9.814		
p	<0.001		0.002		

93.96%, respectively, thus indicating that CEUS has excellent clinical value for the diagnosis of liver cancer.

MVI and the differentiation grade of cancer lesions are important factors that can affect the prognosis and treatment of liver cancer. Efficient protocols to detect MVI and differentiation grades by postoperative pathological examination are inefficient and needs urgent development. Being able to identify MVI and determine differentiation grades by preoperative imaging is of great significance for clinical decision-making and the judgement of patient prognosis. In this study, we identified a positive correlation between positive MVI and an increase in the maximum diameter of liver cancer lesions by >15% in the arterial phase. These data indicate that CEUS has good value for reflecting the MVI of liver cancer, as suggested previously by Morin [26]. This might be because at the time to peak enhancement, the extension indicated the infiltration of inflammatory tissues around the lesions; in addition, inflammation is also known to exert influence on MVI [27–29]. In addition, there were clear differences in enhancement between cancer lesions with various differentiation grades. In the portal phase, lesions with a low/intermediate grade of differentiation showed as low enhancement while lesions with a high grade of differentiation showed as high/equal enhancement. During the delayed phase, 86.96% of lesions with a low/intermediate grade of differentiation showed as low enhancement, while only 55% of lesions with a high grade of differentiation showed as low enhancement. This is because the blood supply of liver cancer lesions with a high grade of differentiation mainly originates from the portal veins or hepatic arteries; in addition, there is usually a large number of hepatic cell cords and hepatic sinusoids, thus resulting in the slow clearance of contrast agent. In contrast, there are many new vessels and arterial fistulas in liver cancer lesions with a low/ intermediate grade of differentiation, thus resulting in a shorter time to enhancement [30, 31]. Thus, physicians can effectively judge the differentiation grades of cancer lesions according to these enhancement features.

This study has some limitations that need to be considered. For example, we only evaluated a small number of patients. Furthermore, the CEUS features of patients with liver disease and unhealthy life habits were not compared to those with liver disease without unhealthy life habits. These limitations need to be addressed in future research.

5. Conclusions

In conclusion, CEUS possesses high clinical value for the diagnosis of liver cancer, judging MVI status, determining the differentiation grades of liver cancer lesions, and can provide reference guidelines for the diagnosis of liver cancer and the evaluation of severity.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

MHL, LH, HML and LZ—designed the study and carried them out; supervised the data collection, analyzed the data, interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of Affiliated Tumor Hospital of Xinjiang Medical University (Approval no. 2020043). Written informed consent was obtained from a legally authorized representative for anonymized patient information to be published in this article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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